

**USE OF PASSIVE ACOUSTICS TO IDENTIFY AND  
CHARACTERIZE SPOTTED SEATROUT SPAWNING HABITAT IN  
TWO MISSISSIPPI ESTUARIES**

**FINAL TECHNICAL REPORT**

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**Eric R. Hoffmayer, Jennifer A. McKinney, Jim S. Franks and Bruce H. Comyns<sup>1</sup>**

**Center for Fisheries Research and Development**

**<sup>1</sup>Department of Coastal Sciences**

**Gulf Coast Research Laboratory**

**The University of Southern Mississippi**

**703 East Beach Drive**

**Ocean Springs, MS 39564**



**GULF COAST  
RESEARCH LABORATORY**  
THE UNIVERSITY OF SOUTHERN MISSISSIPPI

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## ABSTRACT

Spotted seatrout, *Cynoscion nebulosus*, is a highly prized saltwater recreational fish in the Gulf of Mexico. Given that they spawn in estuarine and nearshore waters and are highly exploited, a critical need for their sustainability is to assess and protect spawning habitat. The objective of this study was to use passive acoustics to identify locations of spotted seatrout spawning aggregations in Mississippi waters based on male courtship sounds. The following environmental parameters were measured at sampling sites and used to evaluate spawning habitat: temperature, salinity, dissolved oxygen, depth, current flow, and bottom type. The acoustic survey was conducted within two Mississippi estuaries: Grand Bay (a pristine bay included in the National Estuarine Research Reserve) and Biloxi Bay (a heavily impacted bay) from May to September 2008 and 2009. Spotted seatrout aggregations were heard at nearly three times as many locations in Grand Bay (n=93) compared to Biloxi Bay (n=24). In Biloxi Bay, salinity (>22 ppt) was significantly higher in locations where spotted seatrout aggregations were present, and a positive association with artificial structure was observed. In Grand Bay, stations containing aggregations were in significantly deeper water (> 2.5 m) than stations without aggregations and were often associated with sandy bottom habitat. Additionally, the majority of spotted seatrout spawning aggregations in both estuaries were within close proximity (< 0.4 km) to steep bathymetric relief (1-2 m).

In addition to the mobile survey, long-term acoustic recording systems (LARS) were deployed in each bay during 2009 to determine the diel and seasonal variability of spotted seatrout spawning activity at identified spotted seatrout aggregation sites. The LARS recorded 10 seconds of sound every five minutes from 1600 to 0400 hr. Analysis of the

LARS data indicated that the duration of aggregation courtship calls ranged from 1.25 – 7.4 hours in Biloxi Bay (mean:  $4.4 \pm 0.1$ ) and 0.5 – 7.8 hours in Grand Bay (mean:  $4.8 \pm 0.2$ ). The duration of aggregation calls increased around the full moon phase in both bay systems. Although this study identified spawning habitat within these two estuaries, this research needs to be expanded throughout Mississippi coastal waters to gain a better understanding of critical spotted seatrout spawning habitat within the region.

## INTRODUCTION

Spotted seatrout, *Cynoscion nebulosus*, inhabits estuarine and nearshore Gulf of Mexico waters from the west coast of Florida to the Gulf of Campeche (Mercer, 1984; Pattillo et al., 1997) and is one of the most sought-after saltwater recreational fish species in the southeastern United States. In Mississippi, changes in the inter-annual abundance of this species have become a management issue. In particular, the recreational landings of spotted seatrout have been increasing in coastal Mississippi state waters since 1995 (Fulford and Hendon, 2010). The current spawning potential ratios (SPRs) for spotted seatrout in Mississippi are estimated to be between 8 to 10 % (R. Hendon, Gulf Coast Research Laboratory, unpub. data.), which is well-below the recommended 20% and indicates that this species continues to be overfished (Fulford and Hendon, 2010). Current trends in Mississippi's coastal population growth and increased development, post-Hurricane Katrina, could impact fish species that spawn close to shore through increased fishing pressure and spawning-habitat degradation. This study aims to delineate essential spawning habitat for spotted seatrout.

Spotted seatrout spawn in coastal and estuarine waters (Mok and Gilmore, 1983; Saucier and Baltz, 1993; Brown-Peterson and Warren, 2001; Vanderkooy, 2001; Brown-Peterson et al., 2002), primarily in their natal estuary (Bortone, 2002; Holt and Holt, 2002). Off Texas and along the west coast of Florida, spotted seatrout spawn in estuaries in association with grassbeds near shallow channels (2-4 m) (Brown-Peterson et al., 1988; Walters et al., 2007; Walters et al., 2009). In South Carolina, spotted seatrout spawning has been documented in estuaries near bulkheads (Saucier et al., 1992), and in Georgia estuarine spawning was found near oyster beds (Lowerre-Barbieri et al., 1999). Spotted seatrout spawned in dredged or natural channels within Louisiana estuaries, as well as in passes between barrier islands (Saucier and Baltz, 1993). Spotted seatrout has been reported to spawn within a wide range of water temperature, 19° to 34° C and salinity, 7 to 37 ppt (Brown-Peterson et al., 2002), with optimal salinity ranges apparently related to the hydrographic conditions of a specific area (Holt and Holt, 2002). Prior to this study, scientific documentation of spotted seatrout spawning in Mississippi coastal waters was lacking.

In Mississippi, spotted seatrout reach 50 % sexual maturity at age one, and peak spawning occurs from mid-April to mid-September (Brown-Peterson and Warren, 2001). Spotted seatrout are batch spawners, capable of spawning multiple times throughout the reproductive season (Brown-Peterson and Warren, 2001). Data from an ongoing Mississippi spotted seatrout monitoring study recently documented hydrated females (spawning within 24 hrs) from multiple locations along the Mississippi coast, including three locations within the Biloxi Bay estuary (Hendon and Hoffmayer, unpub. data). A recent otolith microchemistry study indicated that Grand Bay National Estuarine Research Reserve

(NERR) was an important source of spotted seatrout in the eastern portion of Mississippi's coastal waters (Comyns et al., 2008). Tag-recapture studies revealed that spotted seatrout exhibited high site fidelity to Mississippi waters with little movement between embayments (Hendon et al., 2002).

Because of low spawning potential ratios, and young age (age 1 or 2) of hydrated (84%) and running ripe females (94%) collected in a fisheries independent survey (GCRL unpub. data), the population is likely highly susceptible to disruptive recruitment. If for example, oyster beds are primary spawning habitat for spotted seatrout in Mississippi waters, as reported for spotted seatrout in Georgia waters (Lowerre-Barbieri et al., 1999), then the population size may have been reduced since 90% of the oyster beds were destroyed by Hurricane Katrina in August 2005. Obtaining spawning habitat information is vital to the management and sustainability of this species in Mississippi coastal waters.

Most sciaenid (trouts and drums) males produce species-specific courtship sounds with their extrinsic sonic muscles (Connaughton and Taylor, 1994; Figure 1). Females have

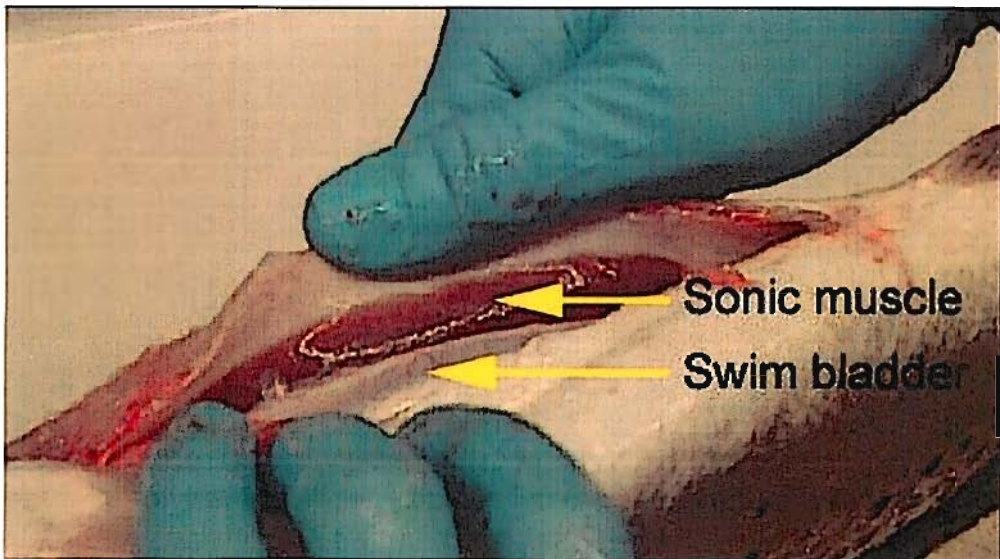


Figure 1. Photo showing the extrinsic sonic muscle of a mature male spotted seatrout. Photo Credit: Florida Fish and Wildlife Research Institute.

no such sound producing capability. These courtship sounds are directly related to spawning activity (Mok and Gilmore, 1983; Saucier et al., 2002; Saucier and Baltz, 2003), and are generally limited to the season and geographic area in which the species spawns (Mok and Gilmore, 1983; Saucier and Baltz, 1993; Connaughton and Taylor, 1994). Because spotted seatrout are soniferous, it is possible to detect their courtship sound production in Mississippi estuaries via passive acoustic telemetry. Passive acoustic surveys are a powerful, non-destructive way to locate sound-producing male spotted seatrout and document the time and place of spawning over large spatial and temporal scales (Mok and Gilmore, 1983; Saucier and Baltz, 1993; Luczkovich et al., 1999). This method has the advantage over traditional sampling in that fish are not caught to determine spawning activity, the fish can be 'sampled' with minimal to no disturbance, and the various biases associated with entrapment gear are avoided. The difficulty inherent in this method is proving that spawning activity is occurring at the same location as the sound production, an issue addressed by Mok and Gilmore (1983) and Saucier and Baltz (1993) who collected freshly spawned spotted seatrout eggs near sound producing aggregations. The aim of this study is to use passive acoustic telemetry to delineate essential spotted seatrout spawning habitat in support of effective management of this valuable resource in Mississippi coastal waters.



## OBJECTIVES

The overall goal of this study was to identify spotted seatrout spawning habitat in Mississippi coastal waters using passive acoustic technology. Individual objectives include the following:

- 1) Identify, characterize, and compare spotted seatrout spawning habitat between Biloxi Bay and Grand Bay estuaries.
- 2) Determine the spatial and temporal variability associated with identified spotted seatrout spawning aggregations.

## METHODS

### *Collaborative Efforts*

This effort was, in great part, a collaboration between project scientists at the Gulf Coast Research Laboratory and their colleagues (S. Lowerre-Barbieri, S. Walters, and J. Bickford) with the Florida Fish and Wildlife Research Institute (FWRI) in St. Petersburg, Florida, who conducted a similar study in Tampa Bay in 2004. We worked closely with our FWRI colleagues to ensure that the experimental design and sampling protocols in the Mississippi were comparable to that for the Tampa Bay study. During the course of the study, two planning sessions were held with our colleagues in St. Petersburg, which included scianid courtship sound recognition training and data analysis methodology. Our FWRI colleagues were also invaluable advisors throughout the study, and assisted with the periodic questions that arose.

### *Sampling Design*

Biloxi Bay and Grand Bay estuaries (Figures 2-5), were chosen for this study based on available data on environmental differences, habitat types, and habitat quality (Biloxi Bay – a heavily impacted bay; Grand Bay - a pristine bay a portion of which includes the National

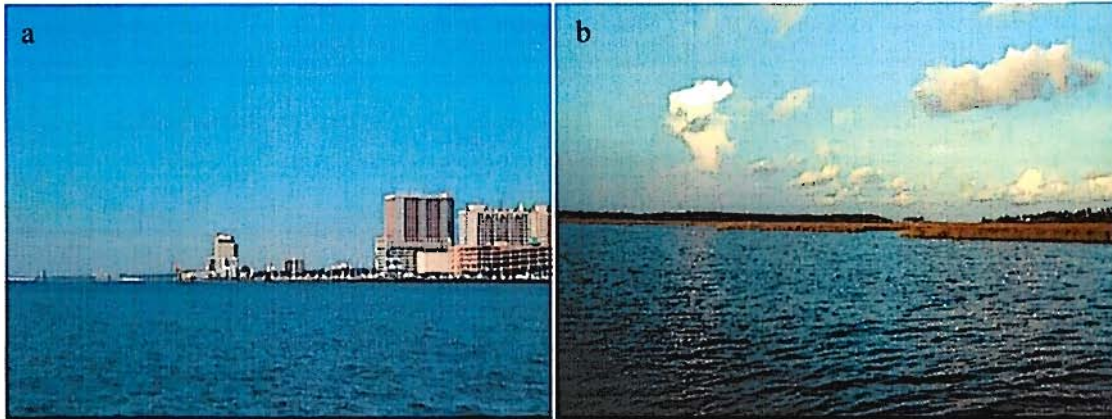


Figure 2. Photos of a coastline area within a) Biloxi Bay estuary, a heavy impacted bay system, and b) Grand Bay National Estuarine Research Reserve, a pristine embayment.

Estuarine Research Reserve). Habitats within the proposed study areas include oyster reefs, seagrass beds, shell beds, sand and mud flats, and marsh edge (Christmas, 1973; Grand Bay NERR, 2007).

A mobile, passive acoustic survey designed to monitor male spotted seatrout sound production was conducted in both study areas during 2008-2009 to identify spawning locations and describe the habitat and environmental conditions associated with those locations. Sampling sites were chosen based on a stratified-random grid system modified from Walters et al. (2007). Each study area was divided into two zones based on geographic and logistic criteria, and each zone was further subdivided into 0.8 km<sup>2</sup> grids (Figures 6 and 7). Grids were further categorized as shoreline or open water. A grid was considered open water if less than 5% of its area comprised of waters less than 1m in depth and adjacent to

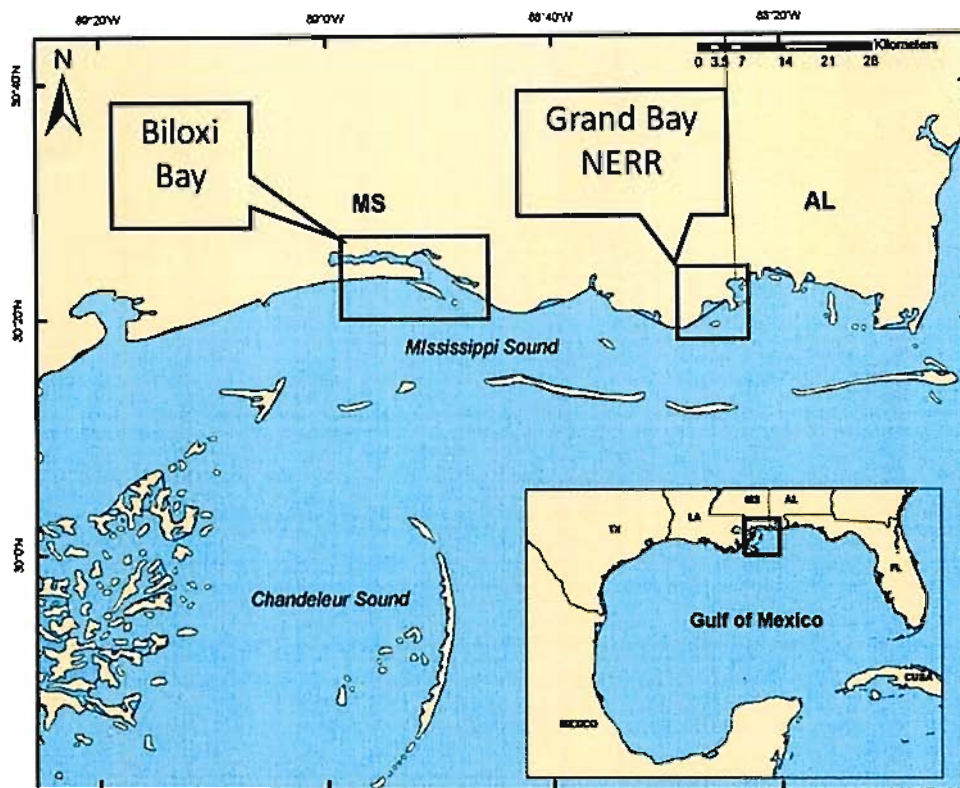


Figure 3. Map showing the Biloxi Bay and Grand Bay study areas in relationship to the Mississippi Sound estuary.



Figure 4. Map showing the Biloxi Bay study area.

land. Six grids were randomly selected for each sampling date with replacement (i.e., returning grids to grid pool to ensure the integrity of the random selection process) in order to account for seasonal variability.

Weekly sampling occurred, and each zone was sampled at least once monthly from May to August. Since spotted seatrout spawning frequencies have been attributed to lunar influences (McMichael and Peters, 1989; Kupschus, 2004; Walters et al., 2007), the selection of zones was rotated over the various lunar phases (i.e. quarter, full, three-quarter, or new moon) during the course of the spawning season. Four survey stations per grid were sampled to ensure representative coverage of the area within each grid, including available substrata. Substrata were characterized as submerged aquatic vegetation (SAV), oyster, structure (including pilings, jetties, bridges, artificial reefs/fish havens, and range markers), channel, sand and mud.



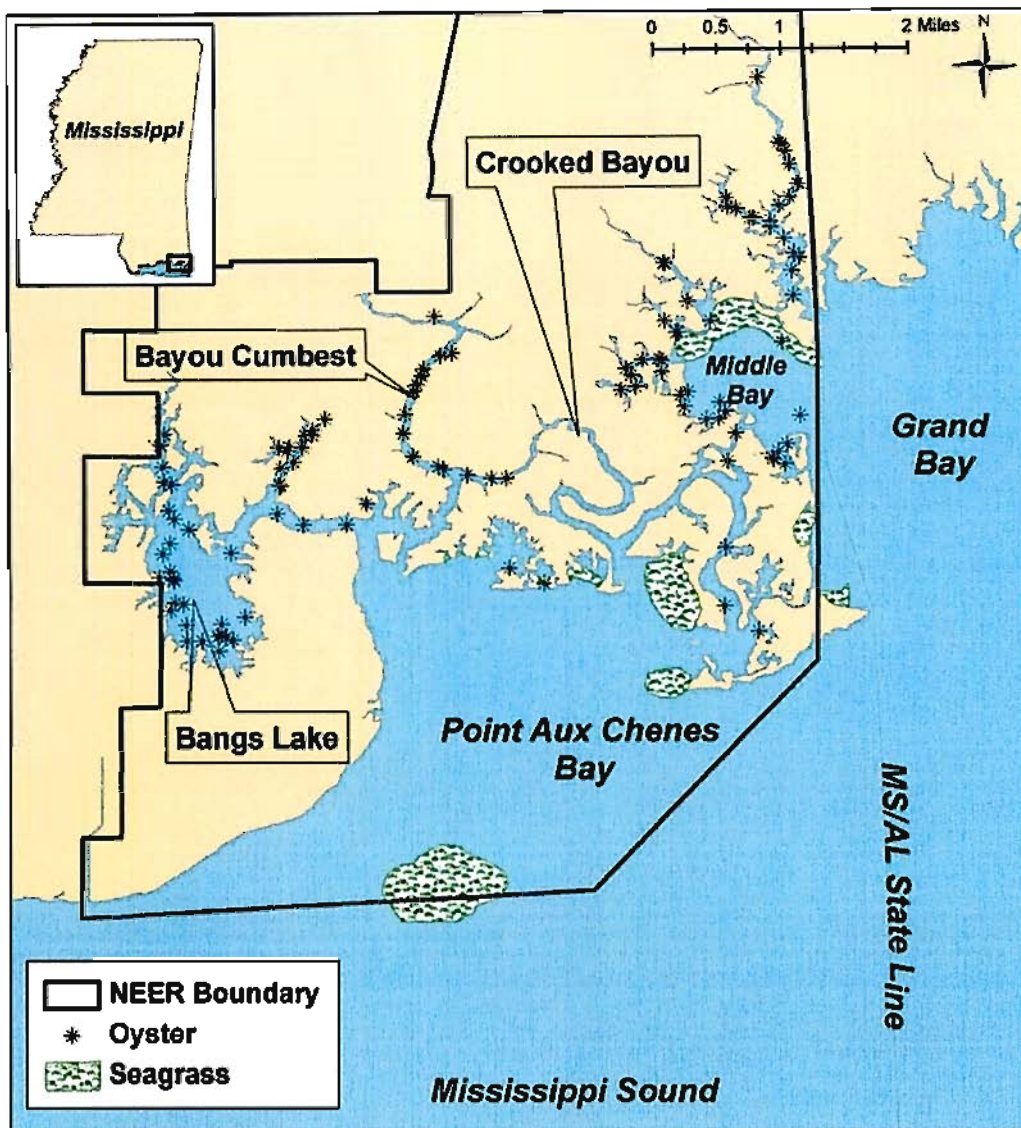


Figure 5. Map showing the Grand Bay study area with the Grand Bay National Estuarine Research Reserve outlined in black.

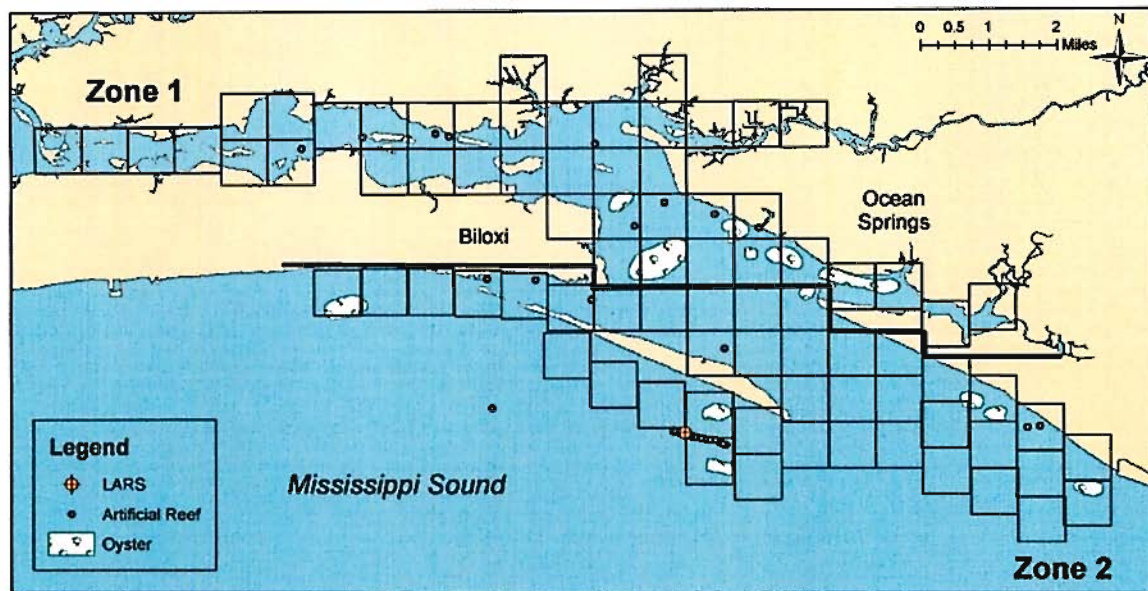


Figure 6. Biloxi Bay study area. Map showing the demarcation between zones 1 and 2 (dark horizontal line), 0.8 km<sup>2</sup> grid system, and locations of oyster beds, artificial reefs and Long-term Acoustic Recording System (LARS).

### *Data Collection*

The mobile, passive acoustic survey was conducted using a mobile hydrophone system (HTI, Model 96-min, frequency range: 2-30 Hz). The survey was conducted between the hours of 1830 to 0200 hr., as this is the peak window of male courtship sound production documented at known spotted seatrout spawning locations in Louisiana (Saucier et al., 1992; Saucier and Baltz, 1993) and Florida (Walters et al. 2007). Geo-referenced habitat data (e.g., SAV, oyster, artificial reef, and bathymetry) for the study areas was provided by the Mississippi Department of Marine Resources and the Grand Bay National Estuarine Research Reserve. These data were imported into ArcMap 9.3 and overlain with a numbered grid system in each bay. Excel random number generator was used to select the grid number to be sampled each week according to the proportion of shoreline vs. open water grids within each sampling zone. In Biloxi Bay, the proportion of shoreline vs. open water grids in zone 1

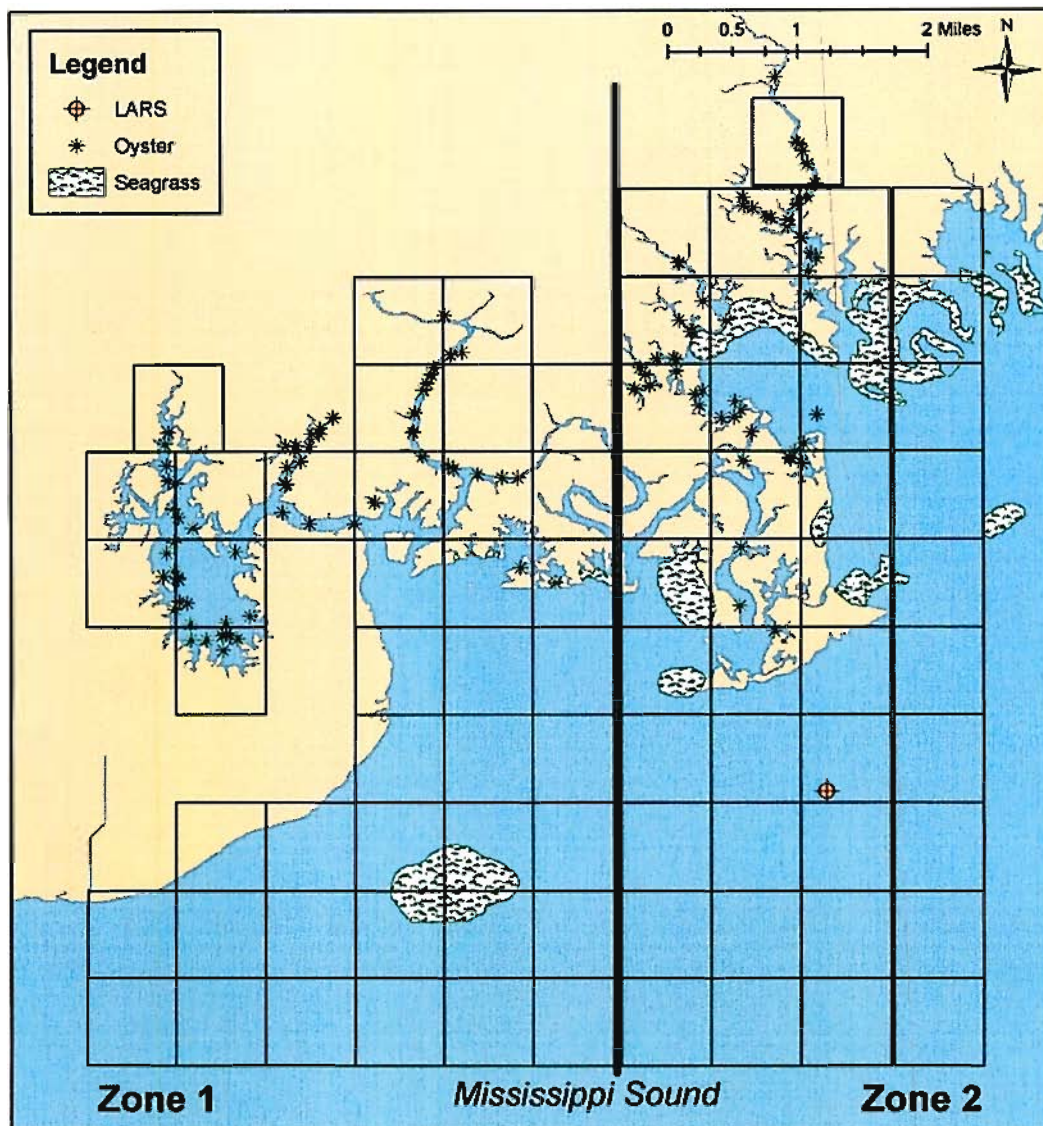


Figure 7. The Grand Bay study area including the NERR. Map showing the demarcation between zones 1 and 2 (dark horizontal line), 0.8 km<sup>2</sup> grid system, and locations of oyster beds, seagrass beds and Long-term Acoustic Recording System (LARS).



and 2 was 5:1 and 4:2, respectively. In Grand Bay, the proportion of shoreline vs. open water grids was 4:2 and 3:3 for zones 1 and 2, respectively.

Hydrophone recordings and environmental data were collected at all stations. Once arriving at a sampling station, the boat motor was turned off, GPS, time, current speed, tide stage (high, low, rising, falling), and depth measurements were recorded. A Yellow Springs Instrument (YSI) meter (Model 85) was used to measure surface and bottom temperature, salinity, and dissolved oxygen.

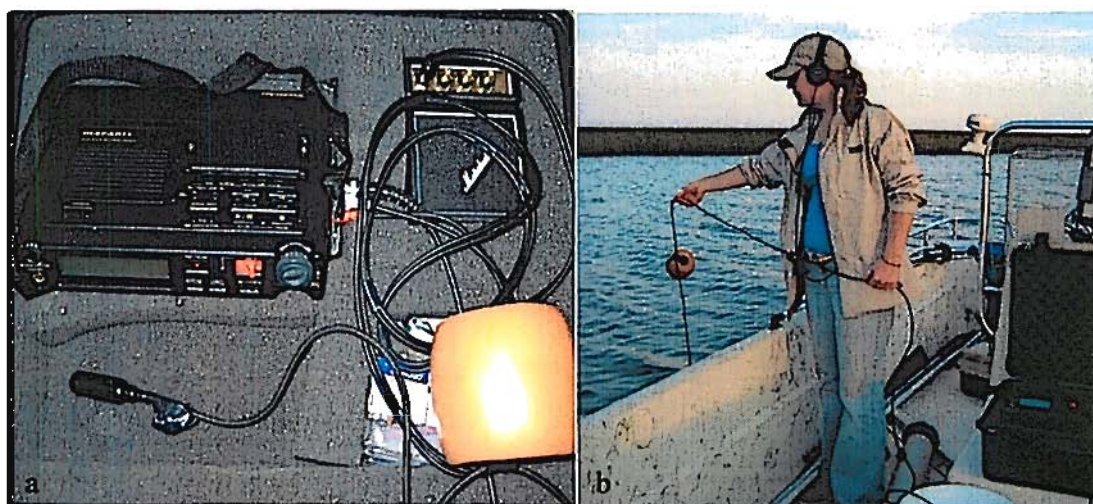


Figure 8. a) The Marantz solid state digital recorder, Marshall mini amplifier, and HTI hydrophone, and b) a demonstration of the deployment of the HTI hydrophone in the field.

A sample of substrata was collected using an Ekman bottom grab, and was recorded as submerged aquatic vegetation (SAV), oyster, channel, sand and mud. Any structure including pilings, jetties, bridges, artificial reefs/fish havens, and range markers were also noted. In order to eliminate the possibility of noise from the boat affecting the behavior of the fish, the hydrophone was not lowered into the water until approximately five minutes after arrival on station. The hydrophone was lowered one meter into the water, and a



miniature Marshall Amplifier was used to listen for all sounds (Figure 8). A 60-second long recording was made using a Marantz solid state digital recorder (Model PMD670).

Headphones were worn if sounds were difficult to detect through the amplifier or if there was excessive ambient noise in the air.

Spotted seatrout males produce distinct courtship calls that have been classified into four major sound types: dual-pulse calls, a long grunt call, multiple-pulse calls, and a stacatto call (Mok and Gilmore, 1983; Figure 9). Those calls can be distinguished from other soniferous fish calls based on pulse duration, repetition rate, and dominant frequency range (Figure 7). The estimated number of spotted seatrout producing sound in this study was

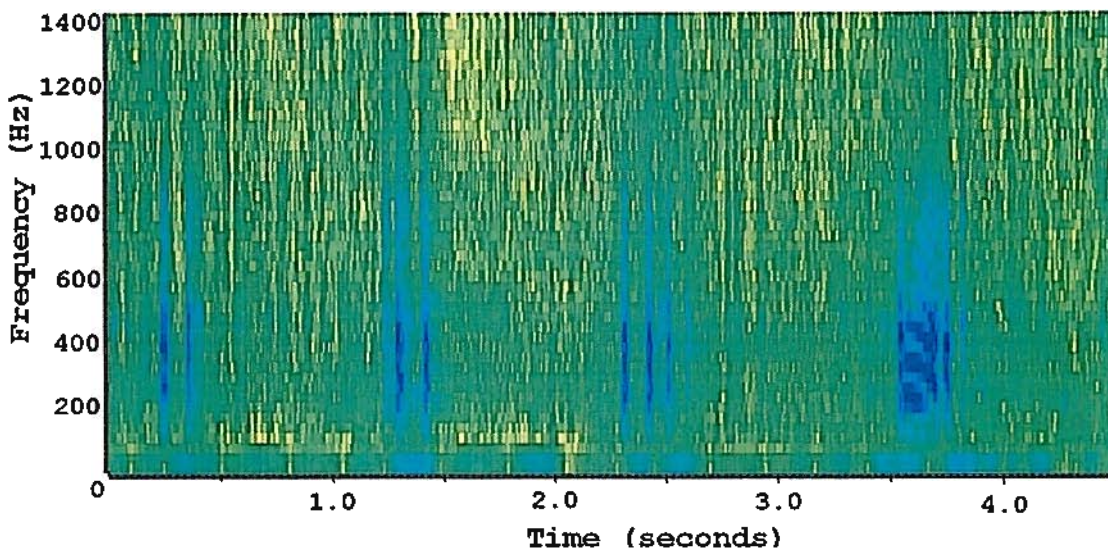


Figure 9. A spectrograph of an individual spotted seatrout courtship call composed of three sets of multiple pulses followed by a long grunt.

categorized as (1) 1-2 individuals, (2) 3-5 individuals, (3) small aggregation (individuals still distinguishable), or (4) large aggregation (individuals not distinguishable). Distance from the hydrophone to the fish was categorized as: “directly on-top of”, “close-by”, or “in the distance” based on intensity of calls (Walters et al., 2009). The “directly on-top of” category

is defined as fish sounds audible through the bottom of the vessel without the aid of the hydrophone; sounds categorized as “in the distance” are quieter, and difficult to hear through the amplifier; “close-by” include a wide range of sounds falling between “directly on-top of” and “in the distance” categories.

In addition to the mobile acoustic survey, in Year 2 of the study fixed-location Long-term Acoustic Recording System (LARS) data loggers (Loggerhead Instruments, DSG-Ocean) were moored at identified spawning locations in both Biloxi Bay and Grand Bay to determine the temporal variability of spotted seatrout spawning activity. The data loggers recorded for 10 second intervals every 5 minutes between the hours of 1600 to 0400 over the duration of deployment from June to September, 2009 (Figure 10).



Figure 10. a) Long-term acoustic recording system (LARS), and b) deployment of the LARS.

All recordings made in the field were reviewed in the laboratory by ear and verified with available known recorded male courtship sounds. A database was developed for the passive acoustic and long-term acoustic data sets. Data were entered weekly and digital recordings were reviewed in the laboratory every three to four weeks. Entered data were quality checked monthly. Sampling locations were mapped with ArcMap 9.3 (ESRI) in order to visualize spatial distribution of spotted seatrout courtship sounds.

#### *Data analysis*

A student t-test was used to determine if there were significant differences in the water temperature ( $^{\circ}\text{C}$ ), salinity (psu), dissolved oxygen (mg/l), depth (m), and current speed (cm/s) in locations where spotted seatrout spawning aggregations were present and absent in both study areas. However, since all variables were not normally distributed and failed to transform, the Mann-Whitney Rank Sum test was used to separate mean ranks. A Chi-Square test with Yates correction for continuity was applied to test differences between expected and actual presence of spawning seatrout to determine if there was a significant preference for a certain substratum. The proportion of all stations sampled with spawning aggregations was used as the expected frequency.

Saucier and Baltz (1993) reported that steep changes in depth are key spawning locations for spotted seatrout. In order to assess if the proximity to steep bathymetric slope was an important factor influencing the distribution of spotted seatrout spawning aggregations in this study, a Chi-squared analysis was conducted to see if there was a higher frequency of spawning aggregations in slope grids than would be expected if grids were used proportionally. Slope grids were characterized as those with a bathymetric change of 1 meter or more within the delineated  $0.8 \text{ km}^2$  grid.

To incorporate all the variables into one analysis, a binomial logistic regression was conducted to determine which environmental variables were most influential to the presence of sound-producing spotted seatrout aggregations. Habitat variables were examined only at stations categorized as “directly on top of” or “close by” because aggregations in the distance may not have been in proximity to the in-situ measurement locations. Variables in this analysis included slope, grid type, substrata type, temperature, salinity, dissolved oxygen, current speed, and tide.

The LARS data were processed using MATLAB (Mathworks) signal processing toolbox, with the DSG lab extension designed by Loggerhead Instruments to allow automated custom-processing of signals. Raw data files were run through a high and low-pass filter (retaining 50-500 Hz) to remove extraneous noise. Sound level was calculated as the Root Mean Square (RMS) and calibrated according to the frequency and hydrophone calibration. To determine start/stop times of spawning aggregations, a conservative threshold was selected by examining the graphs and ensuring the threshold value was above the lowest RMS of all nights. Therefore, if external circumstances, such as rainfall, raised the background noise level, a change in sound intensity would still be detected. A one-hour moving-window was used to determine when the sound level changed above or below the threshold. Start/stop times were manually verified for accuracy. A two-way Analysis of Variance (ANOVA) followed by a Tukey’s HSD pairwise post-hoc test, was conducted to compare the effect of moon phase on the start, stop and duration of spotted seatrout aggregation courtship sound production times, with bay systems (Biloxi Bay and Grand Bay) and time (start, stop, or duration) as the two factors.



## RESULTS

During May to September, 2008-2009, 80.0% (n=64) of the grids in Biloxi Bay and 77.8% (n=81) of the grids in Grand Bay were randomly selected to sample for the presence of male courtship sounds, with 704 stations sampled in total (Biloxi Bay, 356 stations; Grand Bay, 348 stations). The difference in sample size in the two bay systems is attributed to technical and/or weather issues that occurred during the study.

In both bay systems, sound production was detected almost exclusively in the southern portion of the survey area, typically near the mouth of the bay and extending into the Mississippi Sound. The mobile survey in Biloxi Bay detected sound-production in two main areas (Figure 11): 1) south of Deer Island near the Katrina Reef, and 2) waters west of Belle Fontaine Beach. In Grand Bay, spotted seatrout spawning aggregations were detected adjacent to the former Grande Batture's spit (Figure 12).

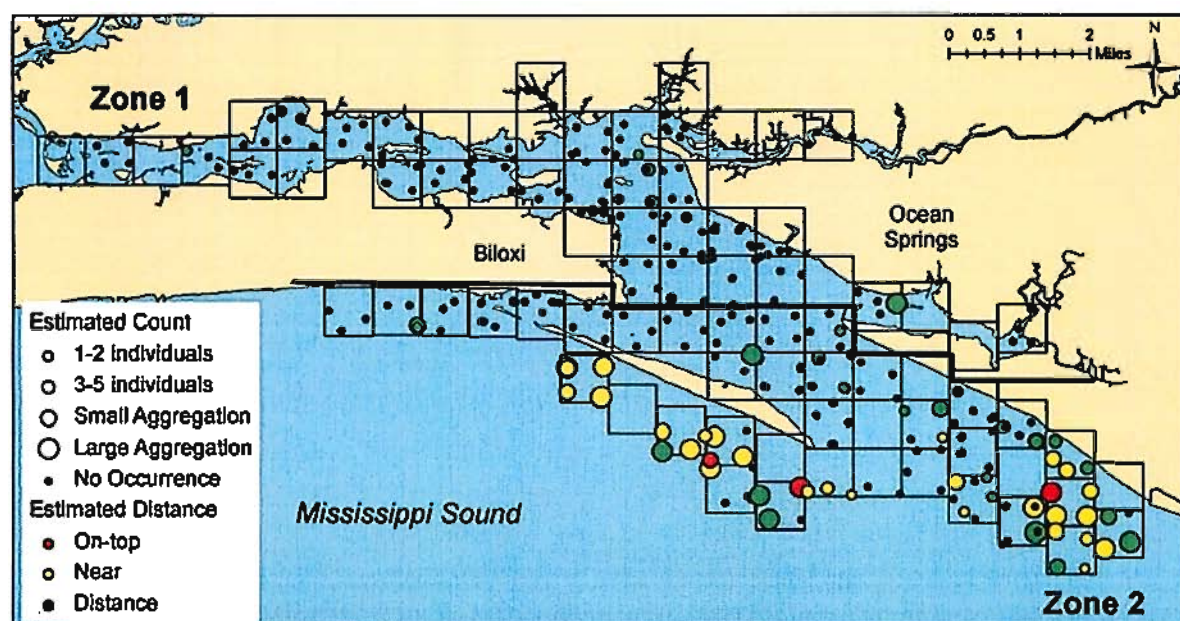


Figure 11. Map depicting locations of spotted seatrout sound production. Color indicates estimated distance from location of sound production. Size of circle indicates estimated number of spotted seatrout producing sound. Black dots indicate no sound detected.

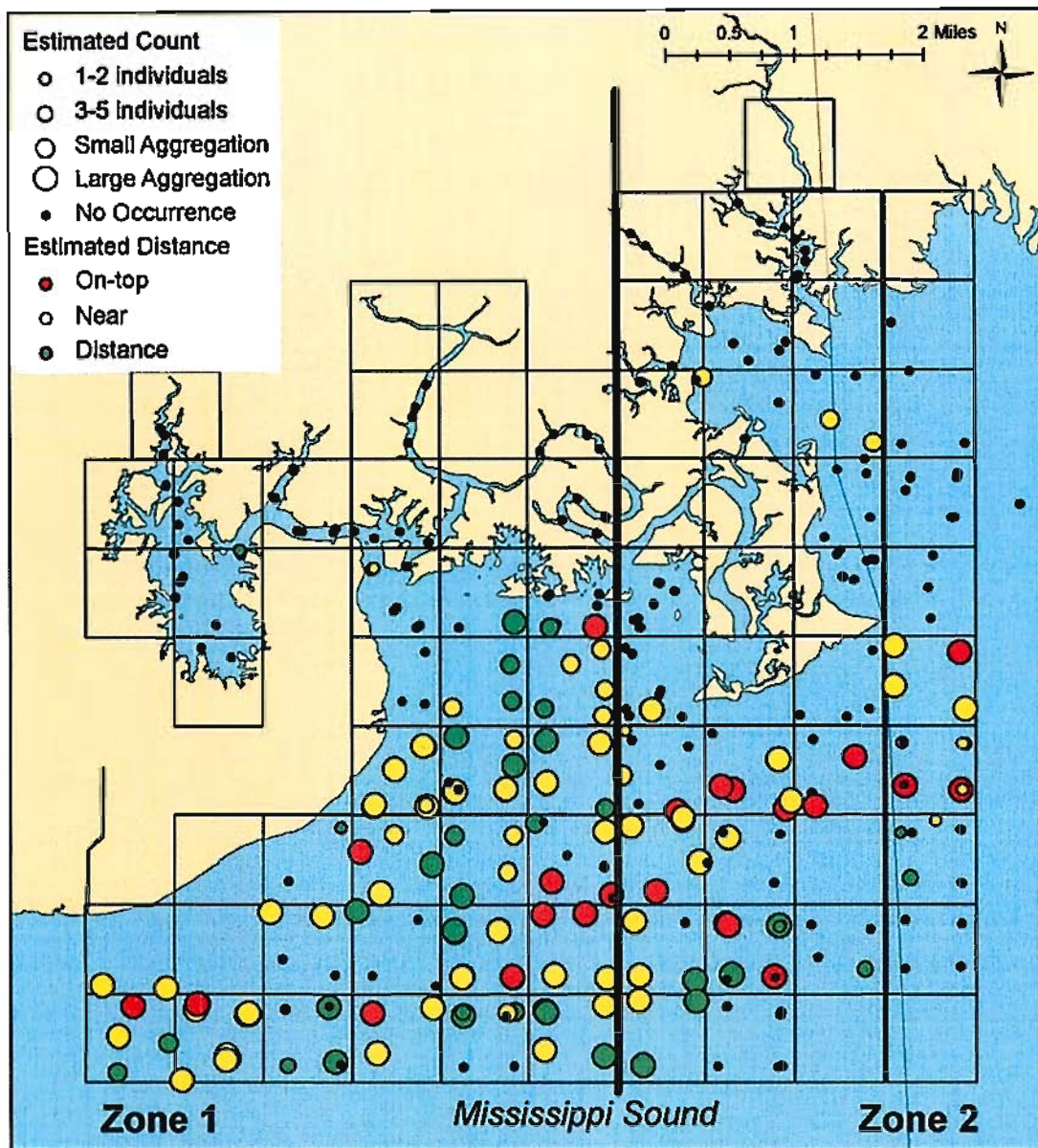


Figure 12. Map depicting locations of spotted seatrout sound production. Color indicates estimated distance from location of sound production. Size of circle indicates estimated number of spotted seatrout producing sound. Black dots indicate no sound detected.

In Grand Bay, spotted seatrout sound-production was detected at more than twice the number of stations ( $n = 152$ , 44%) compared to Biloxi Bay ( $n = 69$ , 19%). In addition, the presence of spawning aggregations was four times greater in Grand Bay ( $n = 93$ , 27%)

compared to Biloxi Bay ( $n = 24$ , 7%). Considerable variability in the use of areas of by large aggregations was observed throughout the duration of the study. Fifty of the 164 grids were sampled at least twice, 19 of those within the same year. Of the grids sampled more than once, only four exhibited spawning activity during each visit, 14 grids exhibited seasonal variability, and no subsequent spawning activity was recorded 29 times within the same grids repeatedly sampled.

### *Habitat Characterization*

Environmental variables were compared within both bay systems at locations where spotted seatrout aggregations were present and absent. Salinity (Mann-Whitney  $U = 7065.5$ ,  $p < 0.001$ ) and current flow rate (Mann-Whitney  $U = 2289.5$ ,  $p = 0.027$ ) were significantly different at locations where spawning occurred in the Biloxi Bay study area, whereas water depth (Mann-Whitney  $U = 19,994$ ,  $p < 0.001$ ) and dissolved oxygen (Mann-Whitney  $U = 19,253$ ,  $p < 0.001$ ) were significantly different where spawning aggregations occurred in Grand Bay study area (Table 1). In Biloxi Bay, spawning activity was identified in areas with significantly higher salinity and lower current flow rate (Table 1). Spawning activity was seldom recorded in the upper reaches of Biloxi Bay, likely because of the considerable fresh water influence in this region (salinities recorded as low as 0.2 ppt; Table 1). Therefore, low salinity stations (ppt  $< 13.0$ ) were removed from further statistical analysis. In Grand Bay, spawning locations were found in significantly deeper water that exhibited higher dissolved oxygen levels than non-spawning locations (Table 1). No significant differences were found among the other environmental variables when comparing presence or absence of spotted seatrout spawning aggregations (all  $p > 0.05$ ).

Table 1. Environmental data collected at all stations in Biloxi Bay and Grand Bay during 2008-2009. Mean, standard error and range are reported. Asterisk indicates variables for which values were significantly different ( $p < 0.001$ ) between locations where spotted seatrout aggregations were present and where they were absent.

	Biloxi Bay		Grand Bay	
	Present	Absent	Present	Absent
Temperature (°C)	30.6 ± 0.1 (28.4 – 32.3)	28.9 ± 0.2 (22.3 – 33.1)	29.4 ± 0.1 (23.6 – 0.4)	29.6 ± 0.1 (22.8 – 0.4)
Salinity (psu)	23.3 ± 0.6* (11.9 – 32.0)	13.6 ± 0.4 (0.2 – 29.6)	23.6 ± 0.4 (14.9 – 32.4)	22.8 ± 0.4 (11.3 – 32.7)
D.O. (mg/l.)	6.5 ± 0.1 (2.1 – 8.3)	6.4 ± 0.1 (1.6 – 10.3)	8.1 ± 0.3* (3.6 – 9.2)	6.4 ± 0.1 (3.1 – 9.0)
Current Speed (cm/s)	10.2 ± 1.2* (0.0 – 80.0)	12.4 ± 0.6 (0.0 – 66.0)	10.7 ± 0.5 (0.0 – 26.0)	9.7 ± 0.8 (0.0 – 67.1)
Depth (m)	2.2 ± 0.1 (0.7 – 4.5)	2.1 ± 0.1 (0.4 – 7.6)	2.5 ± 0.1* (0.5 – 4.3)	1.8 ± 0.1 (0.0 – 4.7)

To determine if there was a preference for a particular substratum as optimal spawning habitat, the proportion of each substratum, i.e., mud ( $n = 392$ ), sand ( $n = 103$ ), sand and mud ( $n = 60$ ), SAV ( $n = 16$ ), oyster ( $n = 62$ ), channel ( $n = 31$ ), and structure ( $n = 17$ ) was compared to the proportion used by the spawning aggregations using a Chi Square analysis. Even though there was an overall significant difference in usage of substrata ( $\chi^2 = 19.2$ ,  $p < 0.001$ ), there were only slight preferences for certain substrata types when compared to the expected habitat use. In Biloxi Bay, artificial structure and mud habitat were minimally preferred, whereas oyster habitat was minimally avoided (Figure 13). In Grand Bay, there was a slight preference for sand and sand/mud habitat and a slight avoidance of oyster and mud habitat (Figure 14).



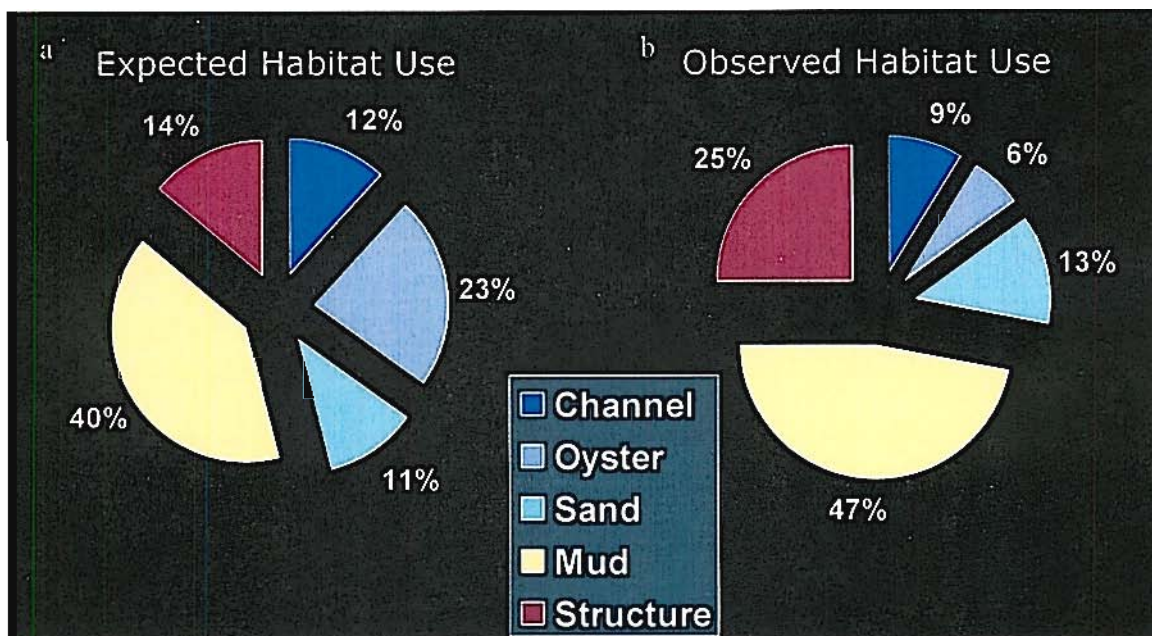


Figure 13. The proportion of a) expected vs. b) observed use of spawning substrata by spotted seatrout in the Biloxi Bay study area.

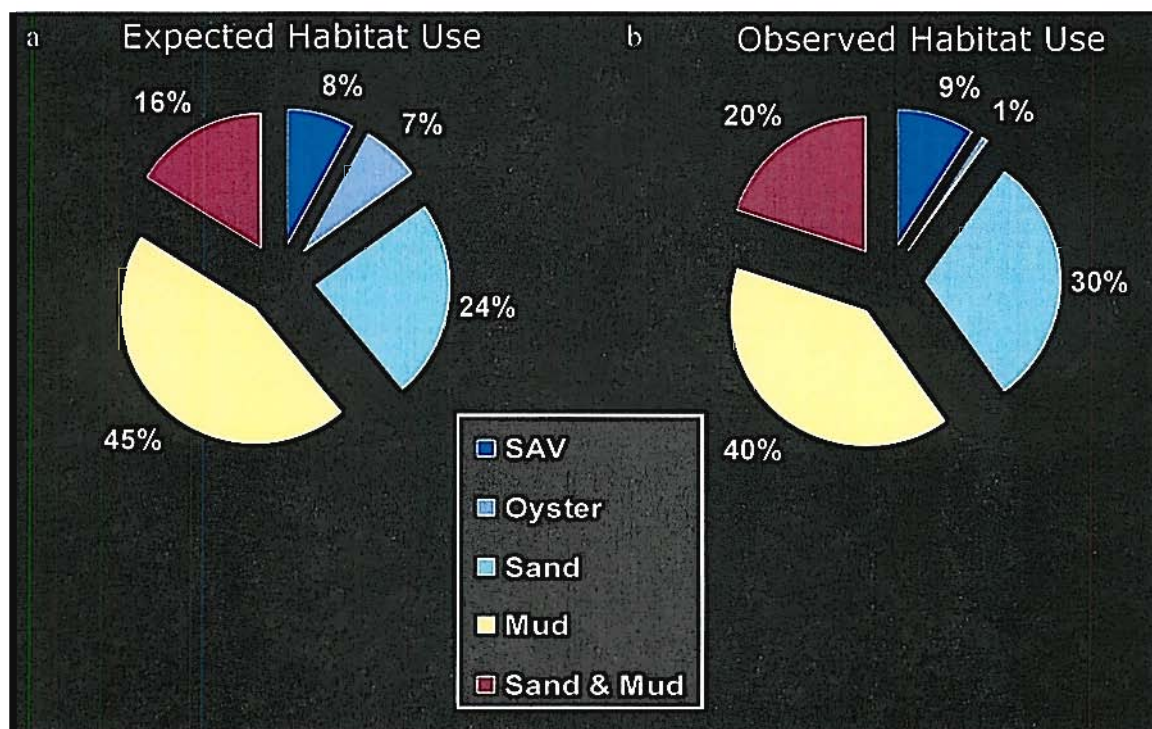


Figure 14. The proportion of a) expected vs. b) observed use of spawning substrata by spotted seatrout in the Grand Bay study area.

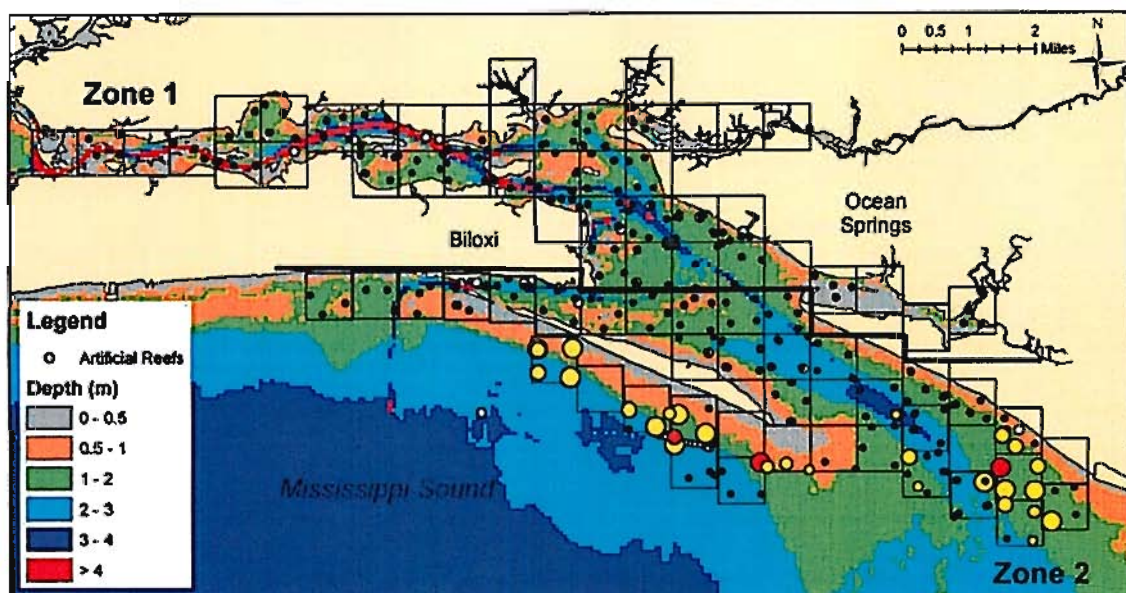


Figure 15. A map of the Biloxi Bay study area depicting the locations where spotted seatrout courtship sounds were detected in relation to water depth. Red circles indicate “on top of” and yellow circles indicate “close by” spawning aggregations. Black circles indicate no spawning sounds were detected.

Due to the lack of a clearly defined preference for particular spawning substrata, the location of depth changes (geographic slope) within the study areas was examined, and slope was found to be a significant factor delineating spotted seatrout aggregation spawning activity in both Biloxi Bay ( $\chi^2 = 303.9$ ,  $p < 0.001$ ) and Grand Bay ( $\chi^2 = 169.9$ ,  $p < 0.001$ ). In Biloxi Bay, 58.0% of the slope grid stations contained spawning aggregations, and only 3% of the non-slope grid stations contained spawning aggregations (Figure 15). In Grand Bay 56.0% of the slope grid stations contained spawning aggregations, and only 12.8% of the non-slope grid stations contained spawning aggregations (Figure 16).



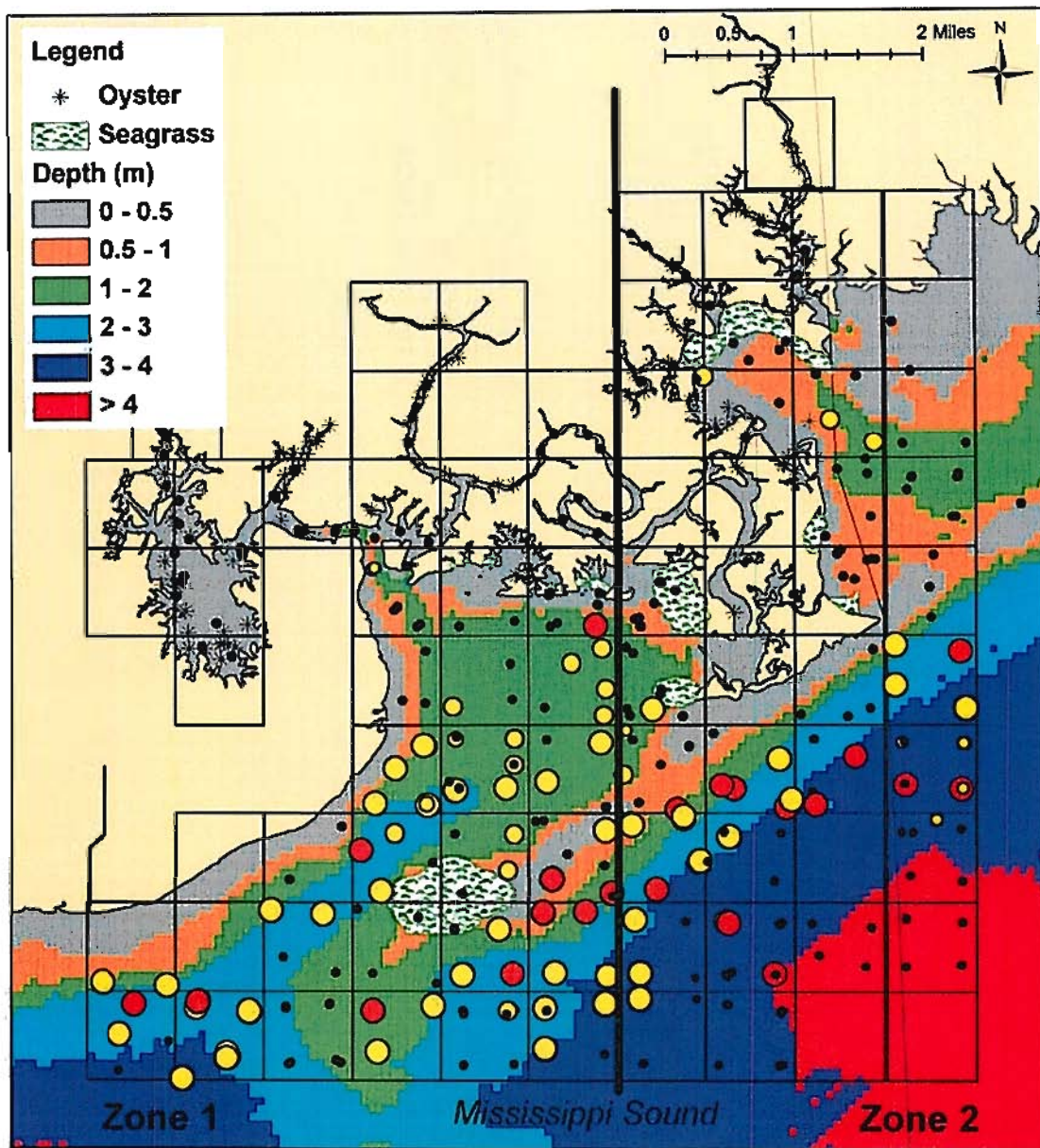


Figure 16. A map of the Grand Bay study area depicting the locations where spotted seatrout courtship sounds were detected in relation to water depth. Red circles indicate "on top of" and yellow circles indicate "close by" spawning aggregations. Black circles indicate no spawning sounds were detected.

The forward-stepwise binary logistic regression analysis converged on a final model that had the highest Nagelkerke  $R^2$ , lowest Log Likelihood test, and a non-significant Hosmer-Lemeshow test (Table 2), describing the best model fit for the data. Slope and high tide were the most significant descriptors ( $p < 0.01$ ) resulting in the detection of spotted seatrout aggregation calls; however, open water grid, temperature, and dissolved oxygen were also significant descriptors ( $P < 0.05$ ; Table 3). Bottom type, current speed, salinity, depth, and low, rising, and falling tide were not included in the model because of the lack of significance ( $p > 0.05$ ).

Table 2. Forward-stepwise binary logistic regression summary evaluation criteria.

Hosmer-Lemeshow $\chi^2$	df	Sig.	-2 Log Likelihood	Nagelkerke $R^2$
11.330	8	0.184	401.480	0.347

Table 3. Forward-stepwise binary logistic regression summary for habitat variable included in the logistic regression equation.

Predictor	B	Wald $\chi^2$	Sig.	Exp(B)
Slope	-2.269	55.693	0.000	0.103
Open Water	0.621	5.413	0.020	1.861
High Tide	-1.226	7.105	0.008	0.293
Temp	-0.244	5.086	0.024	0.784
DO	0.249	4.393	0.036	1.283
Constant	6.751	3.996	0.046	854.494

#### *Temporal variability of spotted seatrout spawning aggregations*

The long-term acoustic recording systems (LARS) deployed on June 8, 2009, recorded for a total of 110 days in Biloxi Bay and 82 days in Grand Bay. Although there was variation in the beginning, end and duration of aggregation courtship sounds between the bay

systems (Figure 17), the mean nightly durations were within one hour of each other. No significant differences in spawning aggregation sound production start, stop, and duration times were evident between Biloxi and Grand Bay ( $F_{1,192} = 3.35$ ,  $p = 0.069$ ), so all the data were pooled. Significant differences were observed between moon phase and mean start time ( $F_{3,188} = 5.12$ ,  $p = 0.002$ ), stop time ( $F_{3,188} = 2.82$ ,  $p = 0.040$ ), and duration ( $F_{3,188} = 5.12$ ,  $p = 0.002$ ) of aggregation calls. The mean times observed during the full moon phase were significantly different from the new moon phases, but not significantly different from the first or last quarter. Significant differences in mean times were also evident between last and new moon phase. During the full and last quarter moon phase, courtship sounds started earlier ( $\sim 1920$ ), ended later ( $\sim 0021$ ), and were longer in duration ( $\sim 5$  hr) than the new moon phase (Table 4). In Biloxi Bay, spawning sounds were detected until the end of the deployment term, although the call duration was reduced (Figure 17). In Grand Bay, sounds of seatrout spawning aggregations sounds could not be detected after September 1<sup>st</sup> due to a masking effect by extraneous noise, which was determined to be red drum based on our familiarity with scianid courtship calls and the concurrent seasonality of red drum spawning (Figure 17).

**Table 4.** Summary of mean start, stop and duration times for spotted seatrout spawning aggregations for the Biloxi Bay and Grand Bay study areas.

Moon phase	Start	Stop	Duration
New	19:36 $\pm$ 6	23:41 $\pm$ 10	4.09 $\pm$ 0.16
First	19:52 $\pm$ 7	0:15 $\pm$ 9	4.34 $\pm$ 0.20
Full	19:20 $\pm$ 6	0:21 $\pm$ 12	5.02 $\pm$ 0.23
Last	19:24 $\pm$ 7	0:14 $\pm$ 10	4.84 $\pm$ 0.16
All combined	19:33 $\pm$ 3	0:07 $\pm$ 5	4.57 $\pm$ 0.10

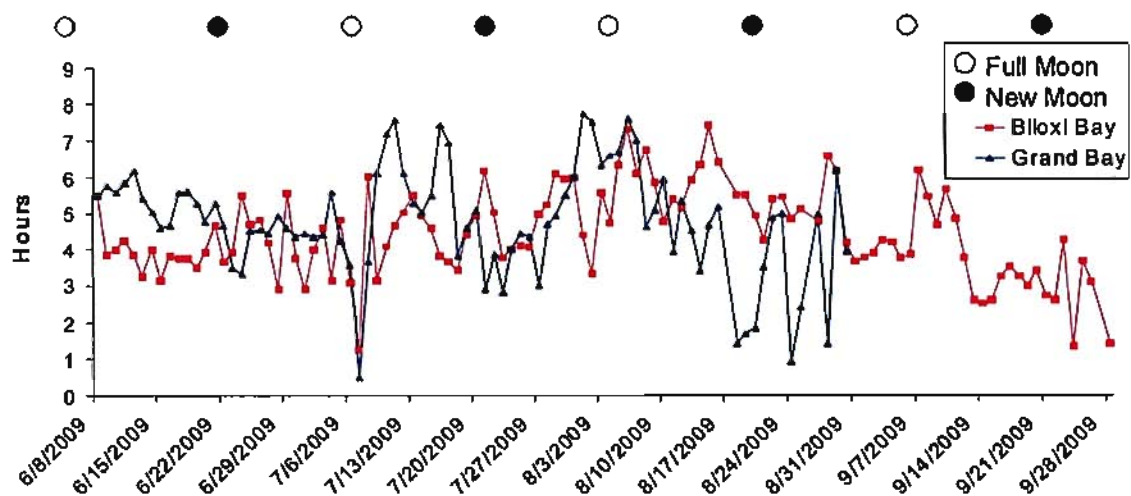


Figure 17: Graphic depicting spotted seatrout aggregation sound production duration in Biloxi Bay and Grand Bay.

## DISCUSSION

### *Geographic distribution and habitat variables associated with spotted seatrout courtship sounds*

Prior to this study, nothing was known about habitat use by spawning spotted seatrout populations in Mississippi coastal waters. Although hydrated females have been captured in the upper reaches of Biloxi Bay (GCRL unpub. data), in this study, male courtship sound production occurred almost exclusively in the southern portions of both bay systems, specifically near the mouths of the estuaries adjacent to the waters of the Mississippi Sound. The mobile survey in Biloxi Bay detected sound-production in two main areas: 1) south of Deer Island near the Katrina Reef, and 2) waters west of Belle Fontaine Beach, in an area that historically was a large oyster reef (J. Franks pers. comm.). In Grand Bay, the majority of areas associated with courtship calls were in close proximity to the once prevalent Grande Batture Islands, which historically, were a series of small islands and a sand spit that extended across a large portion of the mouth of Point Aux Chenes Bay. The Grand Battures have undergone erosion for the past two centuries (Eleuterius & Criss, 1991) and now are only remnant features.

In a similar study conducted in Tampa Bay, spotted seatrout aggregation calls were documented throughout the bay, including its upper reaches (Walters et al., 2009), however, histological analysis determined that seatrout captured at the outer mouth of the bay were dominated by fish with advanced oocyte maturation, whereas those found inside the bay were primarily developing individuals not quite in spawning condition (Lowerre-Barbieri et al., 2009). Spatial distribution of spawning activity may be as important to recruitment success as spawning stock biomass (Begg and Marteinsdottir, 2002). In Mississippi coastal waters,

spatial distribution of spotted seatrout in relation to reproductive stage is poorly known, however, as found in Tampa Bay drumming activity near the mouths of the estuaries may indicate a selection for areas that will support dispersal of larvae into prime areas to enhance larval and juvenile survival.

In this study, male spotted seatrout courtship sounds were significantly more prevalent in Grand Bay than in Biloxi Bay. There are many differences between these two bay systems, including environmental conditions, habitat types, and habitat quality. The Biloxi Bay system was heavily influenced by freshwater input and as a result the majority of the Zone 1 area (Back Bay Biloxi) may have been unsuitable as spotted seatrout spawning habitat due to the low salinity waters. Spotted seatrout spawning aggregations occurred in twice as many locations in Grand Bay as Biloxi Bay. The Biloxi Bay system has been considerably impacted by coastal development and commercial ship traffic that may contribute to the limited use of the bay waters as spotted seatrout spawning habitat. However, it could simply be that there is more available spawning habitat in Grand Bay than Biloxi Bay. It was not surprising to document the extensive spawning activity in Grand Bay, particularly since otolith micro-chemistry analysis showed that Grand Bay was an important source of spotted seatrout for the eastern portion of the Mississippi Sound (Comyns et al., 2008).

Spawning aggregations of spotted seatrout have been documented over a wide range of salinities (Saucier et al., 1992; Saucier and Baltz, 1993; Walters et al. 2009), which may be facilitated by maternally mediated adaptations (Holt and Holt, 2002). As a result, salinity is likely an important driver in explaining differences in reproductive success of seatrout populations throughout the Gulf of Mexico (Brown-Peterson et al., 2002). For example, it



has been shown that low salinities negatively affect spotted seatrout egg buoyancy and diameter (Kucera et al., 2002) as well as juvenile survival (Holt and Holt, 2003). This study found no evidence of spawning (based on courtship sounds) at low salinities ( $< 13$  ppt), and drumming sounds observed during this study were within the range of previously identified salinity regimes (Walters et al., 2009).

In Texas and Florida, spotted seatrout have been documented to spawn near shallow channels (2-4 m) specifically in association with grassbeds (Brown-Peterson et al., 1988; Walters et al., 2007; Walters et al., 2009). Saucier and Baltz (1993) found that in Louisiana, spotted seatrout spawned in dredged or natural channels, as well as in passes between barrier islands. Spawning was also documented near bulkheads in South Carolina (Saucier et al., 1992), and oyster beds in Georgia (Lowerre-Barbieri et al., 1999). In this study, spawning aggregations appeared to be more closely associated with significant changes in bathymetry (0.5 – 1.0 m) rather than any particular substratum type. Furthermore, courtship sound production primarily occurred in areas near a steep gradient in bathymetry that was also associated with SAV. Biloxi Bay lacks SAV, but has extensive oyster beds, artificial reefs, and a large industrial channel that transects the bay. Unlike the Lowerre-Barbieri et al. (1999) study from Georgia, spotted seatrout in Mississippi coastal waters appeared to select against oyster habitat for spawning. Even though, the industrialized channel in Biloxi Bay was extensively sampled, spotted seatrout courtship calls were recorded only on a few occasions. Although it was originally expected that seatrout would utilize SAV and oyster reefs as spawning habitat, these features in Grand Bay existed in waters that are likely too shallow to serve as spawning habitat.

### *Spatial and temporal variability in seatrout courtship sound production*

The timing of nightly courtship sound production documented in this study with both the mobile, passive acoustic survey and with the LARS was similar to observations in South Carolina (Saucier et al., 1992), Georgia (Lowerre-Barbieri et al., 1999), Louisiana (Saucier and Baltz, 1993) and Florida (Walters et al., 2007) waters. The seasonality associated with sound production matched that documented by a gonadosomatic index developed for Mississippi spotted seatrout populations, and indicated a reduction in spawning activity in September (Brown-Peterson et al., 2002). The mobile hydrophone survey and the continuous LARS recording documented a substantial reduction in sound production in September, at which time the study was terminated.

Spotted seatrout spawning frequencies have been reported to be influenced by the lunar cycle (McMichael and Peters, 1989; Kupschus, 2004; Walters et al., 2007), and this was also evident in this study, as well with start time, stop time, and duration of spawning courtship sounds. For example, the longest duration of courtship sounds were heard during the full moon, whereas the shortest duration occurred during the new moon phase.

Spatial and temporal variability of aggregation calls was found in spotted seatrout and other sciaenids in other regions of the Gulf of Mexico. In Georgia waters, it was found that red drum courtship sound production started and ended several hours before red drum sound production in Texas waters (Lowerre-Barbieri et al., 2008). In Tampa Bay, repeated sampling of the same areas determined that habitat use by spotted seatrout aggregations was not consistent throughout the spawning season, and it was suggested that the spatial variability in spawning habitat selection may increase resilience of a population by decreasing impacts caused by catastrophic episodic effects (Walters et al., 2009). In the

present study there was little spatial variability, with the majority of the aggregations occurring in the lower portions of the bay systems; however, there was more temporal variability with moon phase influencing start and stop times, as well as the nightly duration of calling.

#### *Comparison to Tampa Bay study*

Our study design and methodology was adapted from a previous study that identified and characterized spotted seatrout spawning habitat in Tampa Bay, Florida (Walters et al., 2007, 2009). To ensure that the results of the two studies were comparable, the same methods were used, with a reduction in grid size ( $1 \text{ nm}^2$  to  $0.5 \text{ nm}^2$ ) due to the smaller areal coverage of Biloxi Bay and Grand Bay when compared to Tampa Bay. In Tampa Bay, spotted seatrout aggregations were present in only 8% of the stations sampled, with most aggregations occurring in shoreline grids (75%). In Mississippi, spotted seatrout aggregation sounds occurred in 17% of all stations sampled and primarily occurred in open water grids (60%). Important habitat requirements for spotted seatrout spawning in Tampa Bay included elevated dissolved oxygen, water deeper than 1.5 m, and submerged aquatic vegetation (Walters et al., 2009). In Mississippi, elevated dissolved oxygen and waters deeper than 1.5 m were also important; however, due to reduced water clarity and lower salinity regimes, little SAV was available to spotted seatrout in both Mississippi study areas. The prominent habitat feature that was associated with spawning activity in Mississippi was slope, i.e. steep bathymetry changes within the study areas.

The LARS used in this study were similar to the ones deployed in the Tampa Bay study. In Tampa Bay, courtship sound production commenced in mid-May and continued until mid-September (Walters et al., 2009). As for timing of the spawning season, due to equipment complications, our LARS were not deployed until after the start of spawning season (deployment date 6/8/09), therefore we were not able to determine when the courtship sounds began. However, similar to the Tampa Bay study, seatrout aggregation sounds began to decline in mid-September. The range and mean duration of aggregation sounds were less in Mississippi (range = 0.5-7.8 hr; mean = 4.5 hr) than in Tampa Bay (range = 3.0-12.3 hr; mean = 5.9 hr). In both regions, the start time for calls was prior to the sunset nearly three-quarters of the time. In Tampa Bay, call duration increased 1-2 hours around the full and new moons, whereas we observed extended duration during the full moon phase, but a reduced duration during the new moon phase. The timing and nightly duration of courtship sounds appears to be very similar in both areas; however, local populations have adapted to utilize different spawning habitats within the two regions.

#### *Passive acoustic survey*

The mobile passive survey is becoming a standard method for assessing the spatial distribution of sound producing spawning sites, however, it has a few limitations. One major limitation is estimating the distance from sound recording site to the source of the sound production. Spotted seatrout courtship sounds have been recorded up to 150 m away, however, it is extremely difficult to assess the true distance of these sounds because of several factors, including water temperature and depth, bottom type, number of individuals calling, and other ambient noise that can affect the process of estimating distance to the call

source. We consequently used a conservative qualitative scale to estimate the distance, using only data associated with calls that were characterized as “close by” or “on top of” in data analysis.

Methodologies used to estimate spawning locations also have weaknesses. For example, planktonic eggs and larvae can quickly disperse depending on currents, tides, and wind speed from the original spawning location making it difficult to determine their origin. Also, adult fish may not be actively spawning at the time of capture. In conjunction with our acoustic survey, we conducted opportunistic gillnet sets which produced a few specimens of running ripe male and female spotted seatrout, which provided verification that some spawning was taking place at a few of the locations where courtship sounds were detected. Additional acoustic research is needed to gain a better understanding of courtship and spawning dynamics of this species in Mississippi coastal waters.

### *Conclusion*

The results of this study should benefit future management of spotted seatrout resources in Mississippi, help ensure sustainable trout populations, and improve fishing opportunities. This study successfully developed maps and descriptive information on spotted seatrout spawning habitat identified within Biloxi Bay and Grand Bay. Furthermore, this study provides baseline information on the spatial and temporal variability of identified spotted seatrout spawning aggregations within the two bays. Research protocol established in this study is applicable to other valuable sciaenid species (drums and trout) which occur in coastal regions of the Gulf of Mexico. Future research should build upon this study to include the greater Mississippi sound area, in particular, the barrier island passes and SAV associated with the barrier islands.

## IMPLICATIONS

Spotted seatrout is the most popular sport fish in Mississippi coastal waters, and supports a multi-million dollar industry. Potential overfishing and habitat loss and degradation as a result of continuing extensive coastal development could threaten the Mississippi coastal population of spotted seatrout. The Mississippi Department of Marine Resources and the University of Southern Mississippi Gulf Coast Research Laboratory have collaboratively implemented a spotted seatrout stock enhancement program, but this program is only one of several efforts to maintain or increase the population of spotted seatrout in Mississippi.

The year-class-strength of many species is determined during the first few months of life, and so it is essential to conserve both spawning habitat and shoreline nursery habitat. The present study identified critical spawning areas and habitats for spotted seatrout in two Mississippi estuaries; Biloxi Bay and Grand Bay, and developed benthic habitat maps of the areas. Spawning did not occur throughout most of the study areas but was restricted to particular locations with characteristic water depths, salinities and bottom types.

These findings of the proposed research will have application in future management of spotted seatrout which may include future designations of seatrout spawning habitat as areas requiring special protection. In addition, by knowing where a large proportion of spawning occurs in the two bays, future management strategies can incorporate this information with water flow patterns in Mississippi Sound and associated estuaries to determine to which nursery areas spotted seatrout larvae will be passively transported.

Effective fisheries management strategies need to take into account all life history stages. Understanding the locations of spawning activities and associated habitats, such as

provided through this study, is paramount to ensuring sustainable stock levels, particularly with regards to increased coastal development.

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